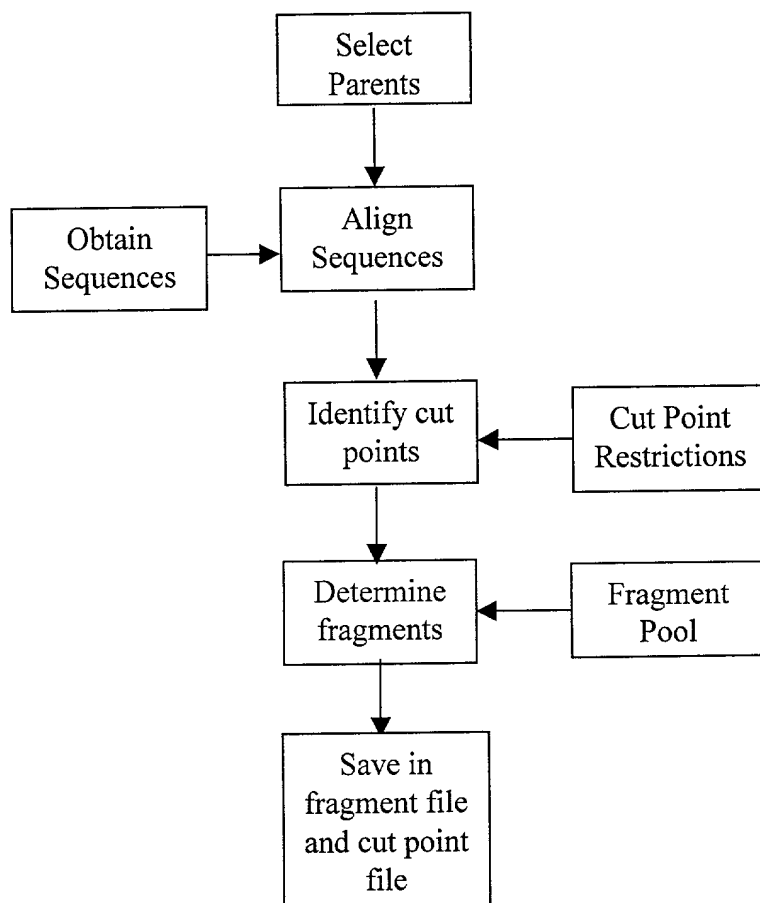


## Determining Possible Fragments Based On Experimental Restrictions



**FIG. 1A**

## Determining the Schema Disruption Profile for a Structure

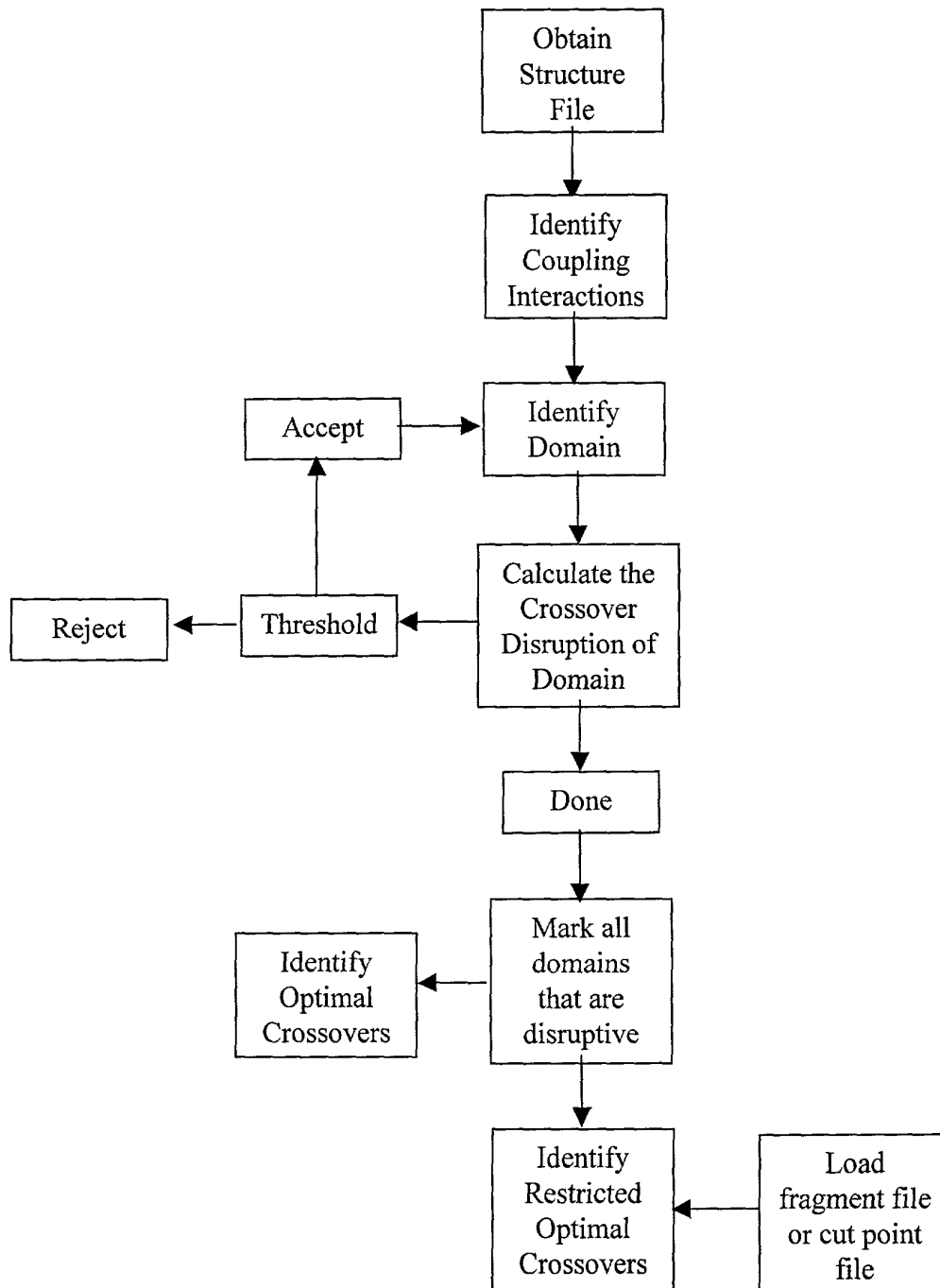
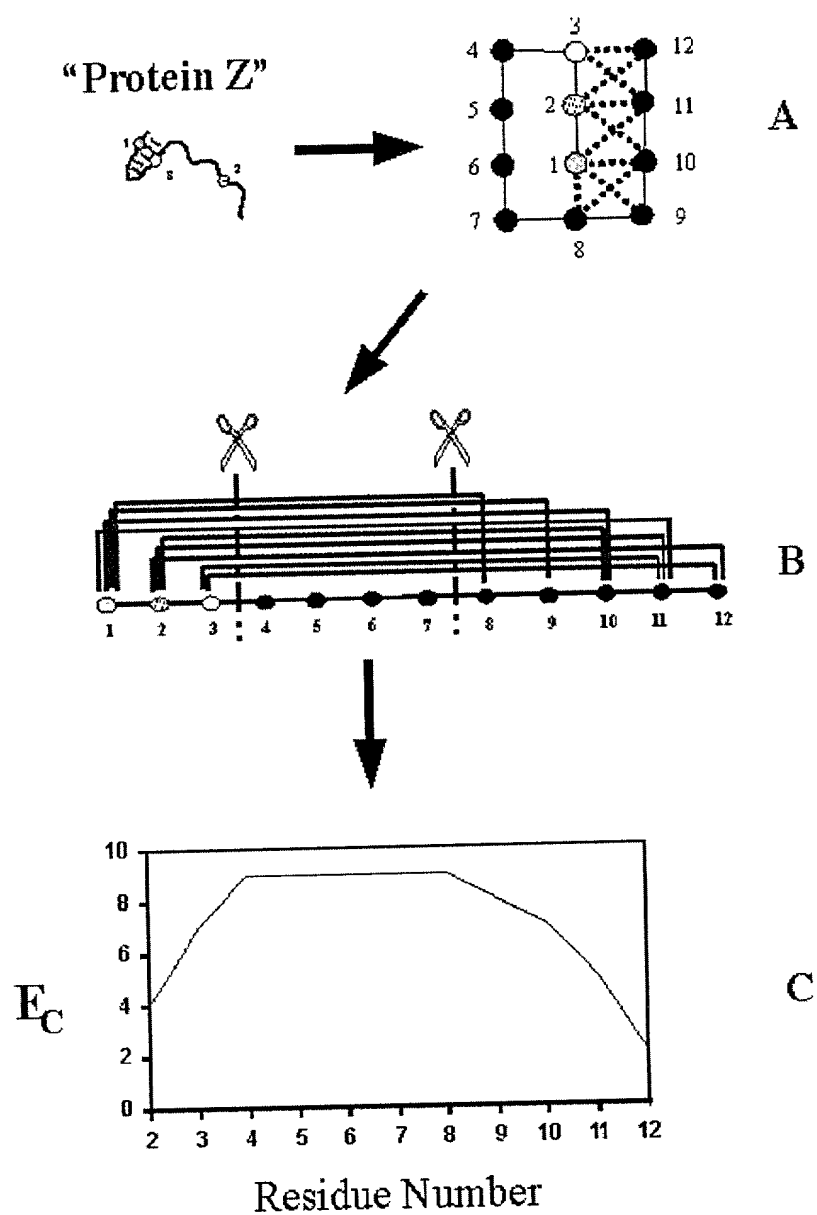


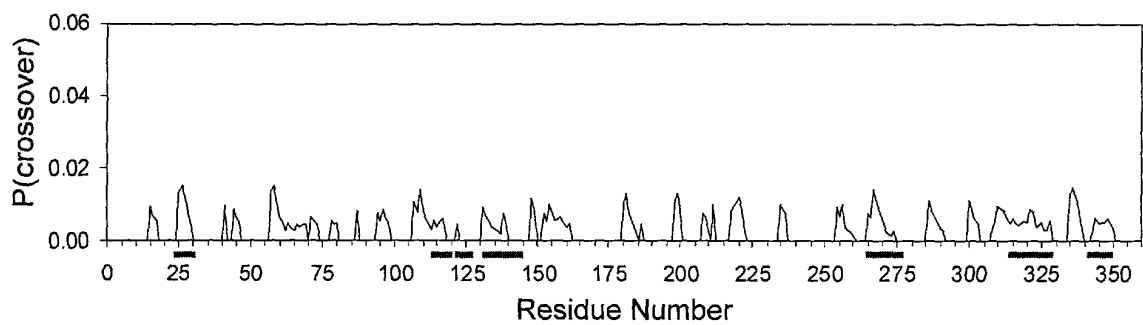
FIG. 1B



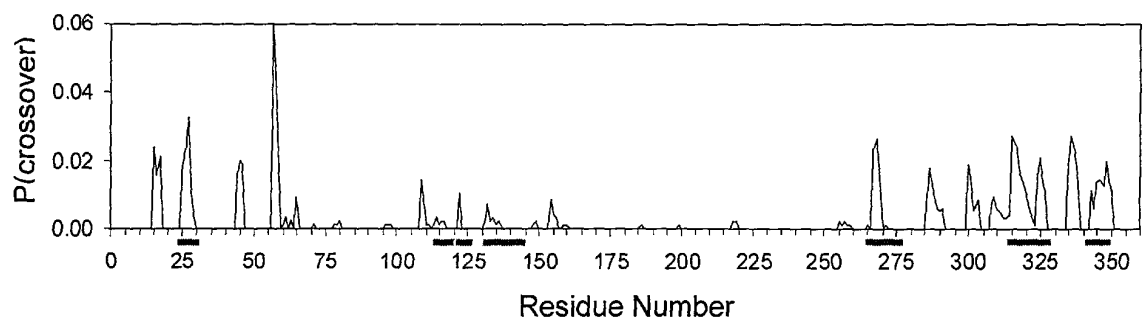
1	1	T	P	V	S	E	K	Q	L	A	E	V	V	A	N	T	I	T	P	L	M	K	A	Q	S	V	P	G	M	A	V	A	V	I	Y	O	G	K	P	H	Y	Y	T	F	G	K	A	D	I	A		
2	1	A	K	T	E	Q	Q	I	A	D	I	V	V	N	N	T	I	T	P	L	M	Q	E	Q	A	I	P	G	M	A	V	A	I	I	Y	E	G	K	P	H	Y	Y	T	F	G	K	A	D	I	A		
3	1	T	K	L	T	E	L	Q	V	A	T	I	V	N	N	T	I	T	P	L	M	L	E	K	Q	A	I	P	G	M	A	V	A	I	I	Y	E	G	K	P	H	Y	Y	T	F	G	K	A	D	I	A	
4	1	Y	A	R	G	E	A	P	L	T	A	A	V	D	G	I	I	Q	P	M	L	K	E	Y	R	I	P	G	M	A	V	A	V	L	K	D	G	K	A	H	Y	F	N	Y	G	V	A	N	R	E	S	
1	51	N	K	P	V	T	P	Q	T	L	F	E	L	G	S	I	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q	
2	51	N	H	P	V	T	E	Q	T	L	F	E	L	G	S	V	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q	
3	51	G	R	P	V	T	E	Q	T	L	F	E	L	G	S	V	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q	
4	51	G	R	P	V	S	E	Q	T	L	F	E	I	G	S	V	S	K	T	L	T	A	T	L	G	A	Y	A	A	V	K	G	F	E	L	D	D	K	V	S	Q	H	A	P	W	L	K	G	S	A		
1	101	W	Q	G	I	R	M	L	D	L	A	T	Y	T	A	G	G	L	P	L	Q	V	P	D	E	V	T	D	N	A	S	L	L	R	F	Y	Q	N	W	Q	P	Q	W	K	P	G	T	R	L	Y		
2	101	W	R	G	I	S	L	L	H	L	A	T	Y	T	A	G	G	L	P	L	Q	I	P	G	D	V	T	D	K	A	E	L	L	R	F	Y	Q	N	W	Q	P	Q	W	T	P	G	A	K	R	L	Y	
3	101	F	D	G	V	T	M	A	E	L	A	T	Y	S	A	G	G	L	P	L	Q	F	P	D	E	V	T	D	S	N	D	K	M	R	T	Y	R	H	W	S	P	V	Y	P	A	G	T	H	R	Q	Y	
1	151	A	N	A	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	E	A	M	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A		
2	151	S	N	A	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	E	A	M	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A		
3	151	S	N	A	S	I	G	L	F	G	H	L	A	A	N	S	L	G	Q	P	F	E	E	Q	L	M	S	Q	T	L	P	K	L	G	L	H	T	Y	I	Q	V	P	E	S	A	I	A	N	Y	A		
4	151	S	N	P	S	I	G	L	F	G	H	L	A	A	N	S	L	G	Q	P	F	E	E	Q	L	M	S	Q	T	L	P	K	L	G	L	H	T	Y	I	Q	V	P	E	S	A	I	A	N	Y	A		
1	201	W	G	Y	R	D	G	K	A	V	R	V	S	P	G	M	L	D	A	E	A	Y	G	V	K	T	N	V	Q	D	M	A	R	N	W	V	M	A	N	M	A	P	E	N	V	A	D	A	S	L	K	Q
2	201	W	G	Y	L	E	G	K	P	V	H	V	S	P	G	Q	L	D	A	E	A	Y	G	V	K	S	S	V	I	D	M	A	R	N	W	V	M	A	N	M	A	P	E	N	V	A	D	A	S	L	K	Q
3	201	W	G	Y	K	D	G	K	P	V	R	V	T	L	G	M	L	G	E	E	A	Y	G	V	K	S	T	S	Q	D	M	V	R	F	M	Q	A	N	M	D	P	E	S	L	G	N	D	A	L	K	E	
4	201	Y	G	Y	K	E	D	K	P	V	R	V	T	P	G	V	L	A	E	A	Y	G	I	K	T	G	S	A	D	L	L	K	F	T	E	A	N	M	G	Y	Q	.	G	D	A	A	L	K	T			
1	251	G	I	A	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	E	G	S	D	S	K	V	A	L	A	A	L	P	V	A	E		
2	251	G	I	E	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	E	G	S	D	S	K	V	A	L	A	A	L	P	V	A	E		
3	251	A	I	I	A	S	Q	S	R	Y	F	Q	A	G	D	M	F	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	E	G	S	D	S	K	V	A	L	A	A	L	P	V	A	E		
4	249	R	I	A	L	T	H	T	G	F	Y	S	V	G	D	M	T	Q	G	L	G	W	E	S	Y	A	Y	P	L	T	E	Q	A	L	L	A	G	N	S	P	A	V	S	F	Q	A	N	P	V	T		
1	301	V	N	P	A	P	P	V	K	A	S	W	V	H	K	T	G	S	T	G	G	F	G	S	Y	V	A	F	I	P	E	K	Q	I	G	I	V	M	L	A	N	T	S	Y	P	N	P	A	R	V		
2	301	V	N	P	A	P	A	V	K	A	S	W	V	H	K	T	G	S	T	G	G	F	G	S	Y	V	A	F	I	P	E	K	Q	I	G	I	V	M	L	A	N	T	S	Y	P	N	P	A	R	V		
3	301	L	V	P	A	Q	P	A	V	R	A	S	W	V	H	K	T	G	A	T	N	G	F	G	A	Y	I	V	F	I	P	E	E	K	N	L	G	I	V	M	L	A	N	K	N	Y	P	N	P	A	R	V
4	299	F	A	V	P	K	A	M	G	E	Q	R	L	Y	N	K	T	G	S	T	G	G	F	G	A	Y	V	A	F	V	P	A	R	G	I	A	I	V	M	L	A	N	R	N	Y	P	I	E	A	R	V	
1	351	E	A	A	Y	H	I	L	E	A	L	Q																																								
2	351	K	A	A	W	R	I	L	E	K	L	Q																																								
3	351	Q	A	A	Y	D	I	L	Q	A	L	R																																								
4	349	K	A	A	H	A	I	L	S	Q	L	A																																								

1	Enterobacter cloacae	P05364 (X03866)
2	Citrobacter freundii	P05193 (X07274)
3	Yersinia enterocolitica	P45460 (X63149)
4	Klebsiella pneumoniae	Q48437 (X77455)

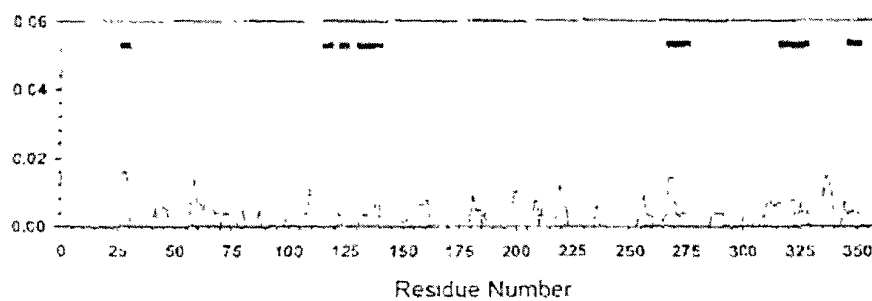
FIG. 3



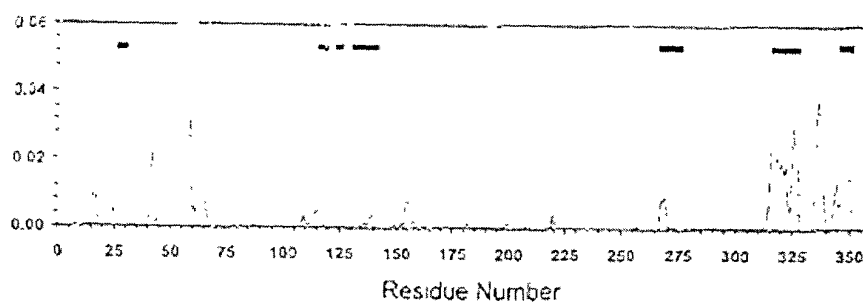
**FIG. 4A**



**FIG. 4B**



**FIG. 4C**



**FIG. 4D**

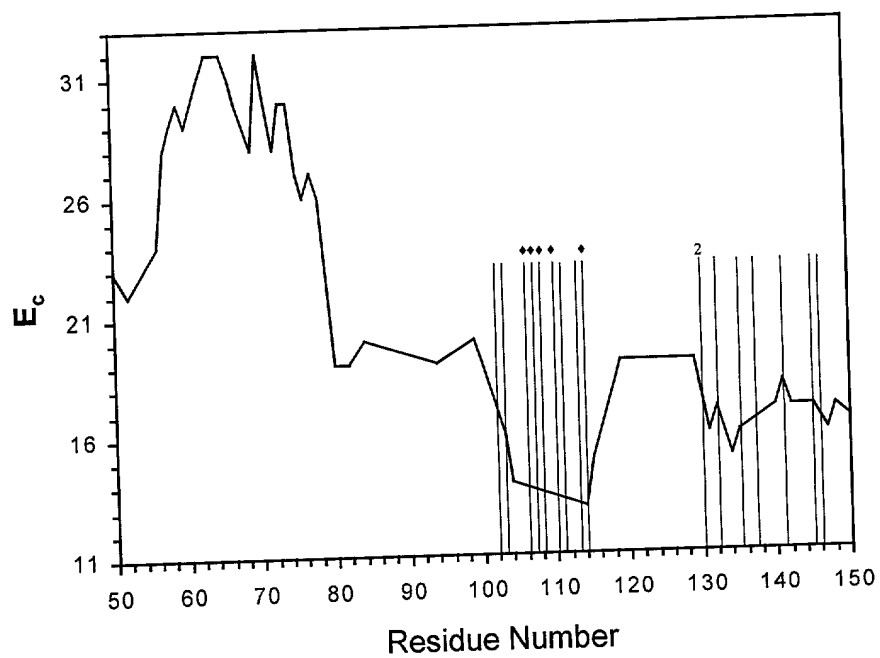


FIG. 5

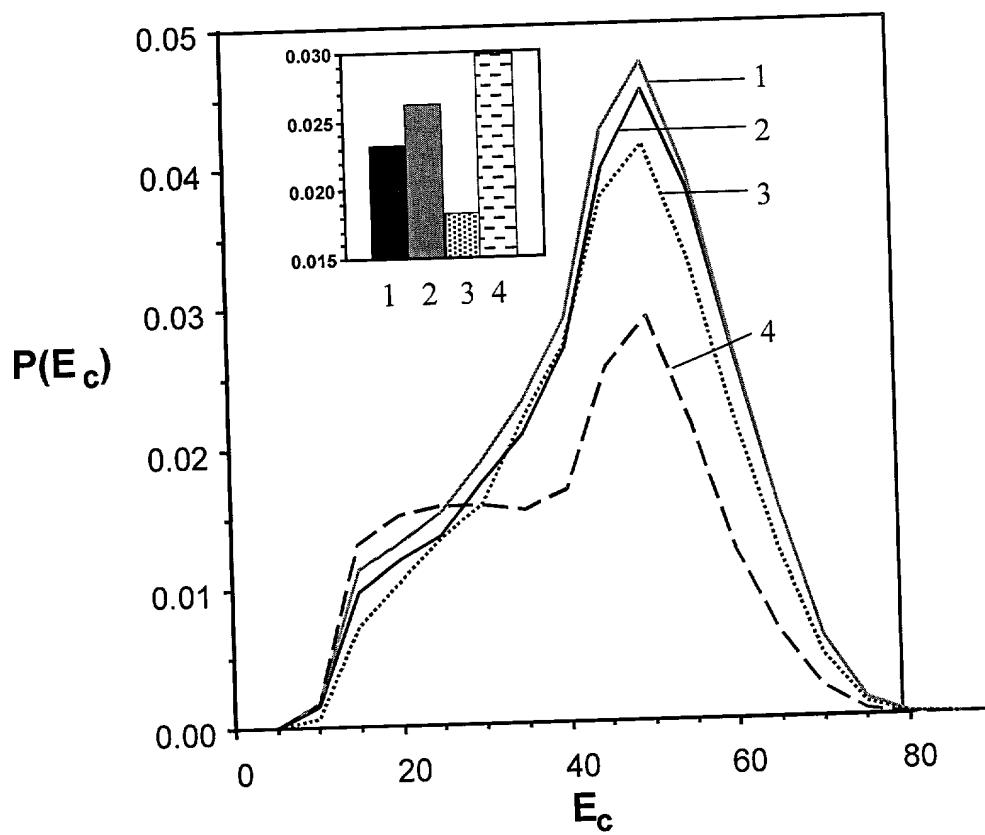


FIG. 6

FIG. 7

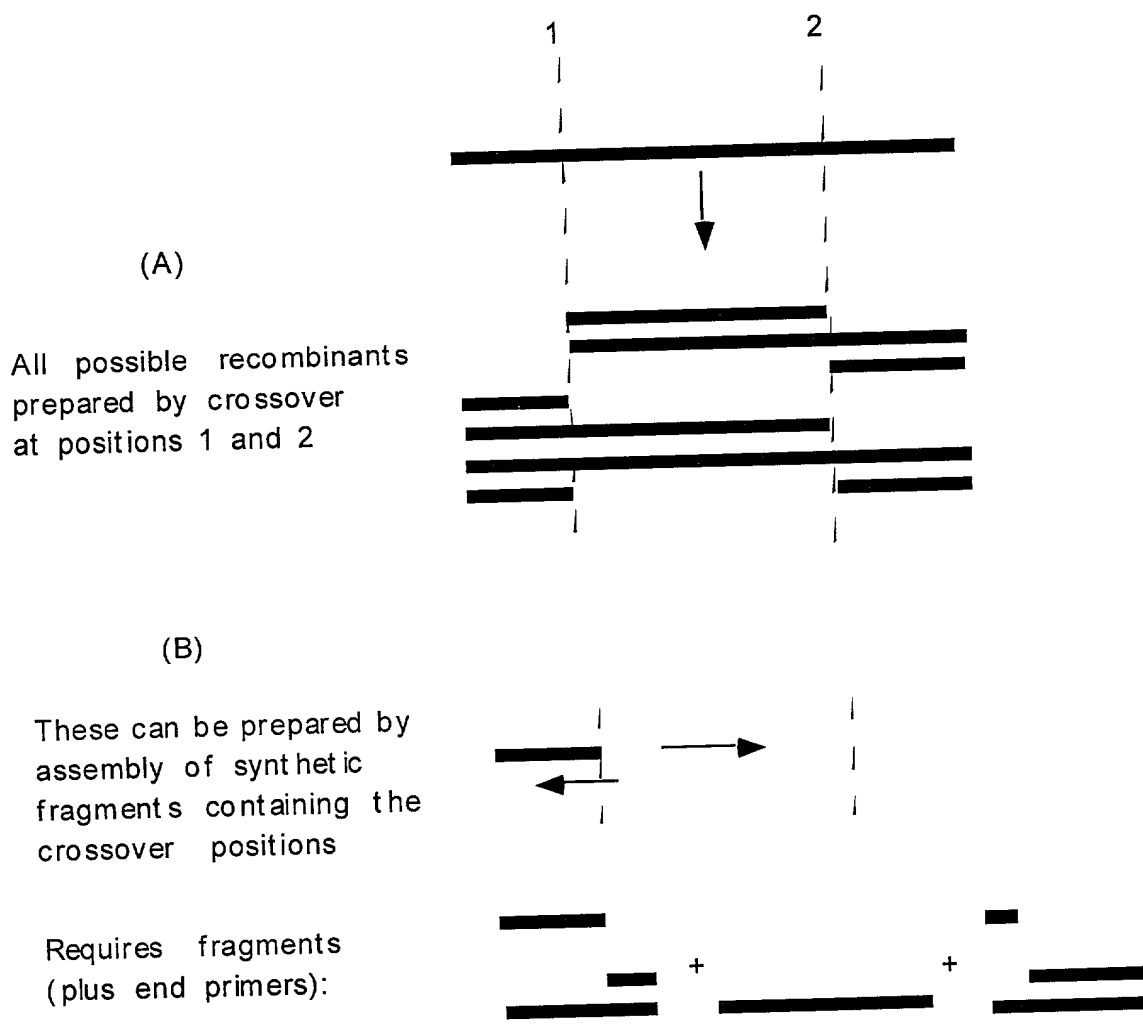


FIG. 7

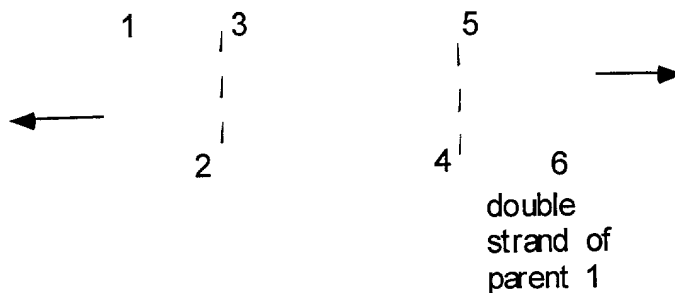


[illegible]

(A)

Prepare the fragments by  
PCR with primers: perform  
reactions with primers 1+2,  
3+4 and 5 +6,

and do same for other parent(s).



(B)

Reassemble fragments in a pool, by PCR with 1+ 6

**FIG. 9**

(A)

Prepare crossover primers  
designed to have crossovers  
at designated positions (2  
primers for each position).



(B)

Fragment parent genes and PCR reassemble in the presence of the  
crossover primers to promote recombination at designated positions

**FIG. 10**

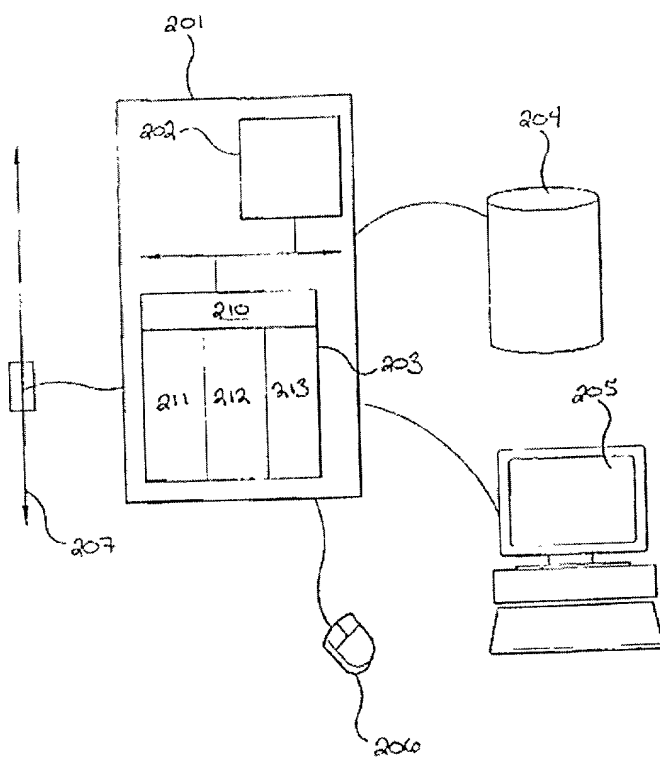
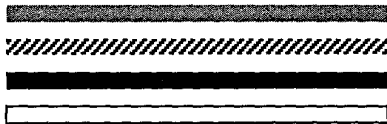


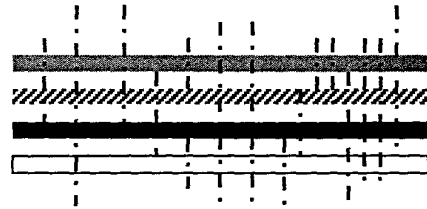
FIG. 11

## Recombinant search algorithm

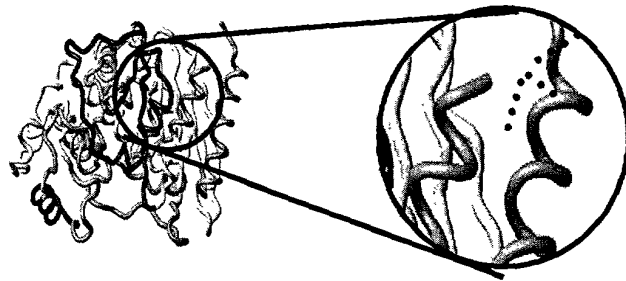
1. Align parent sequences with template structure



2. Determine all possible crossover points according to sequence identity algorithm



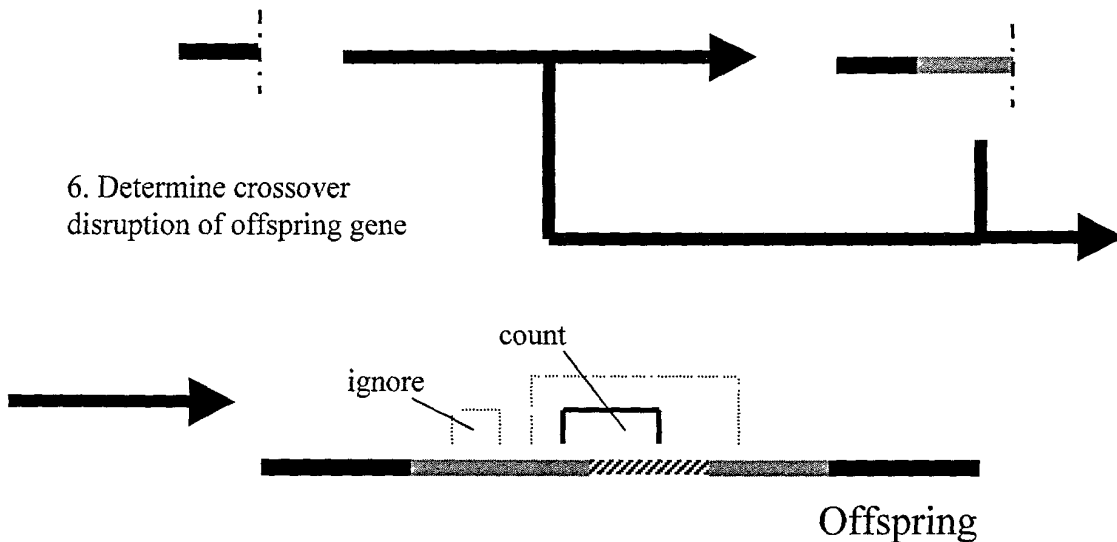
3. Calculate coupling matrix



4. Pick start parent at random and copy to offspring until a possible cut point is reached

5. Pick random number, if less than  $p$ , copy random new parent until next cut point is reached.

6. Determine crossover disruption of offspring gene



**FIG. 12**

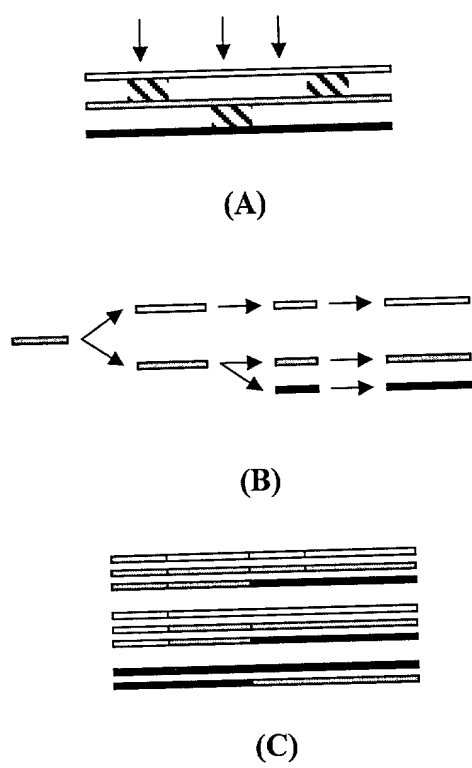
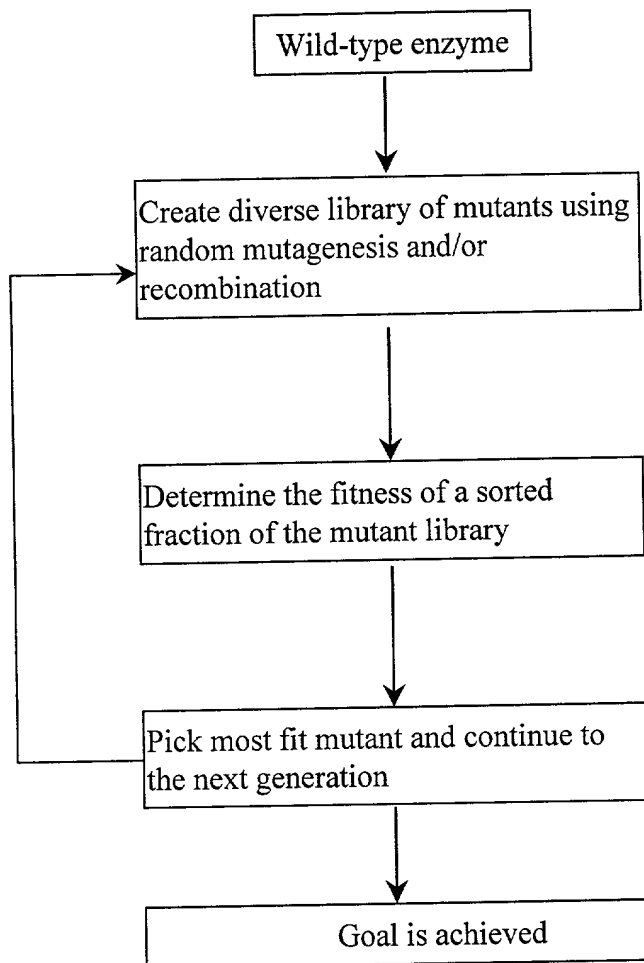
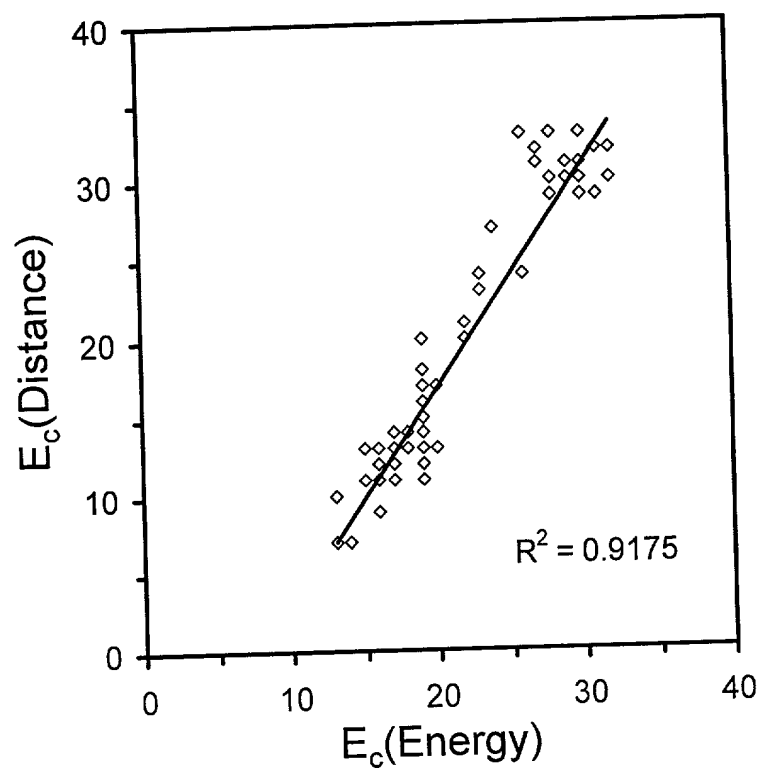


FIG. 13

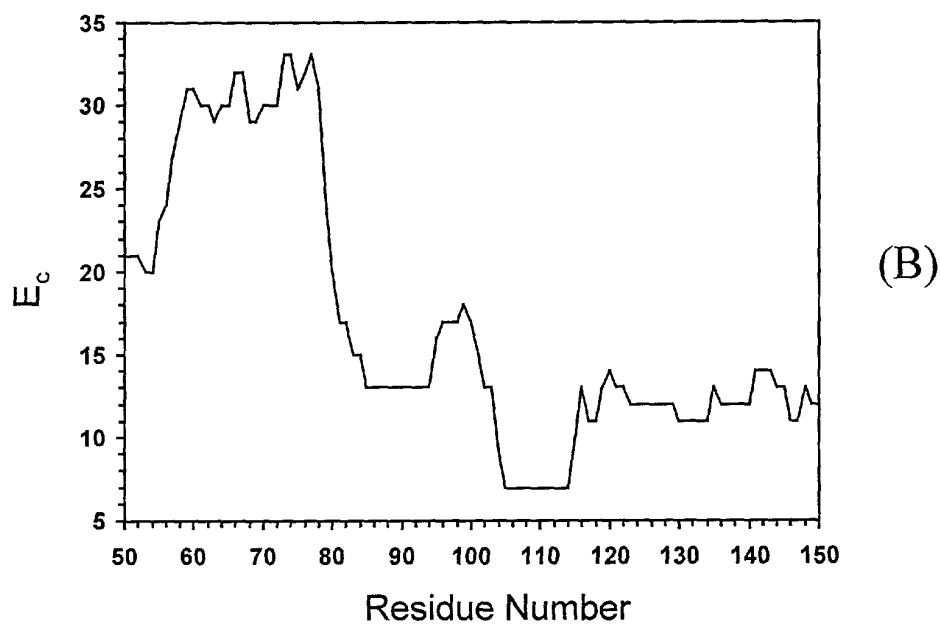
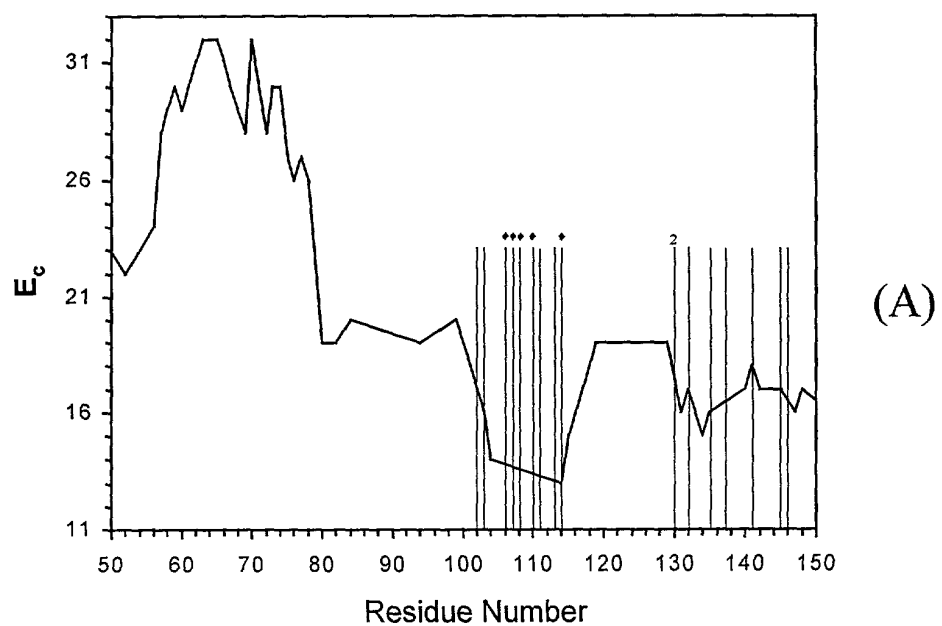
# DIRECTED EVOLUTION ALGORITHM



**FIG. 14**



**FIG. 15**



**FIG. 16**



T06250" 592E9860



(A)

Experimental Data:

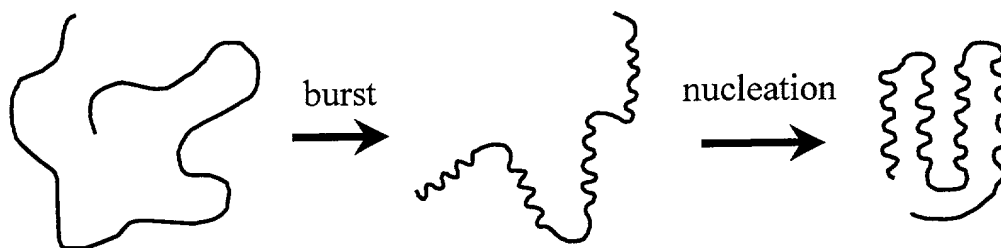
	wt	wt-insert	1	2
Tm(dC)	52	55.2	n.d.	54.3
Tm(dC)	49.5	53.3	44.5	52.5
t1/2	12.1	2586	-	87.5
t1/2	53	138	4	308

(B)

Calculations:

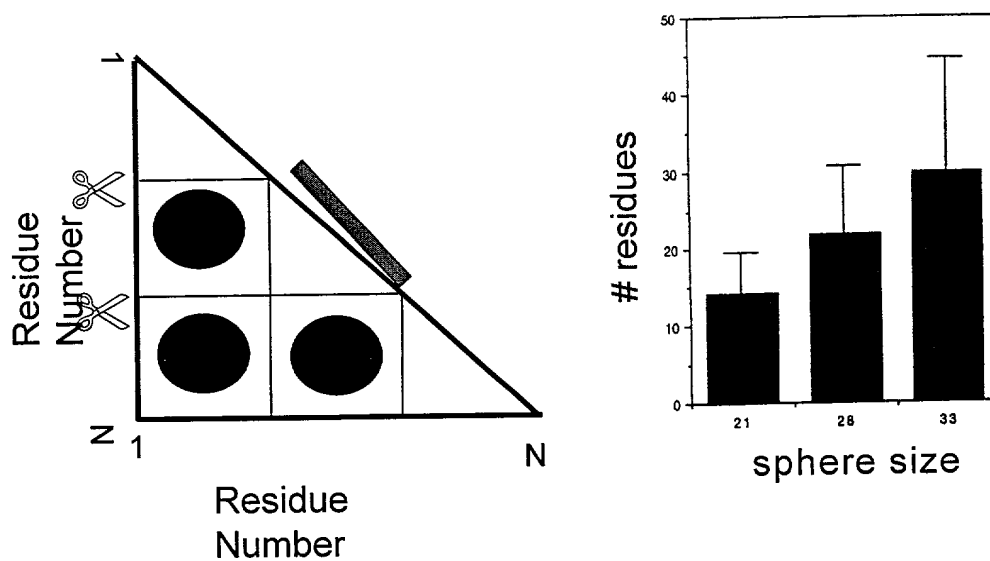
	All schema		Fragments		Z-score	
	av	stdev	1	2	1	2
Ec	19.260	4.090	10.770	8.124	-2.076	-2.723
Ec*	0.006	0.002	0.014	0.005	4.838	-0.857

FIG. 17



**FIG. 18**

The contact map shows residues that are distant (black) and residues that are close (white). If a given segment, XXXXXXXXXX, folds an above average number of residues into a given sphere size, then it is compact.



**FIG. 19**

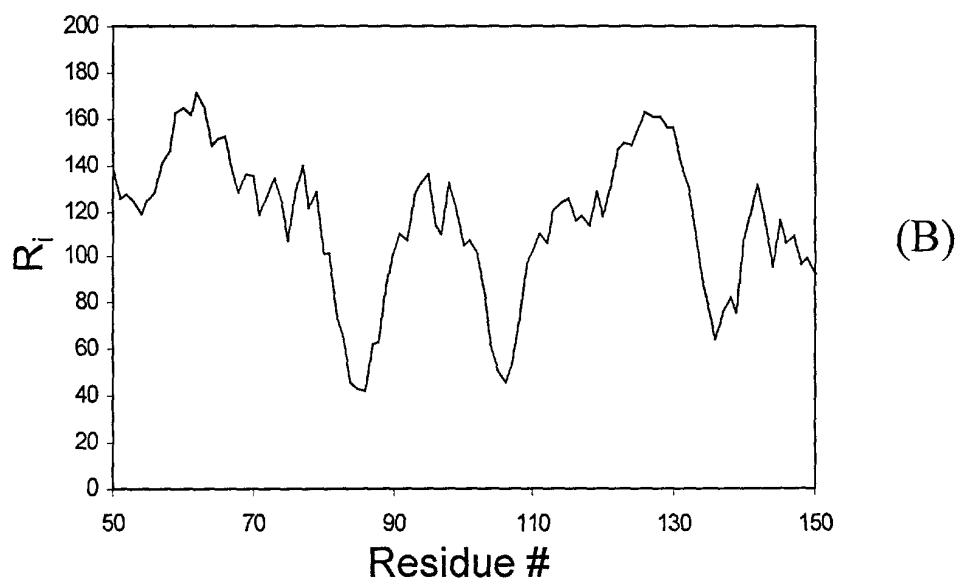
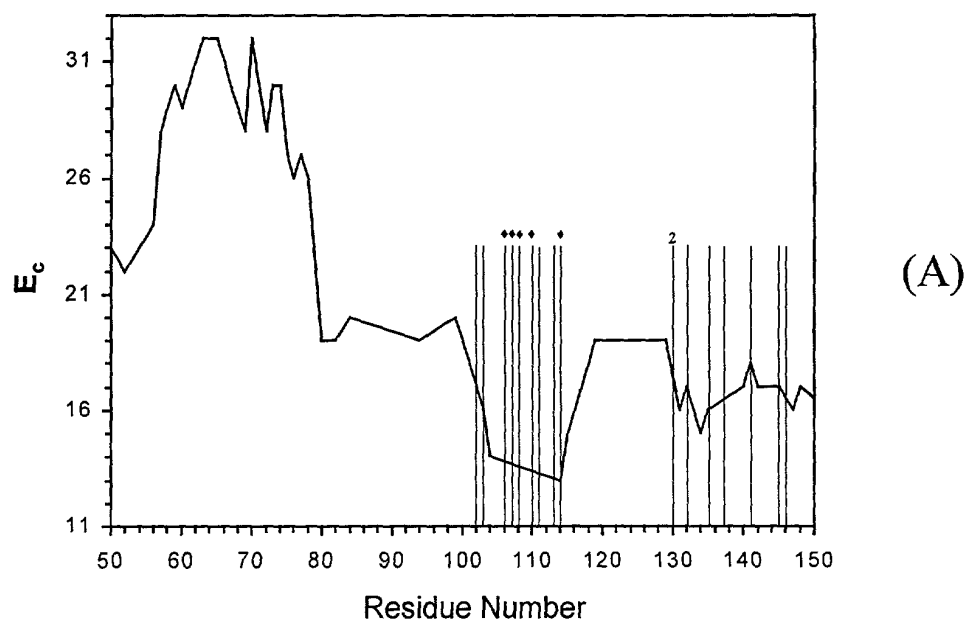


FIG. 20

FOE250" 994E9860

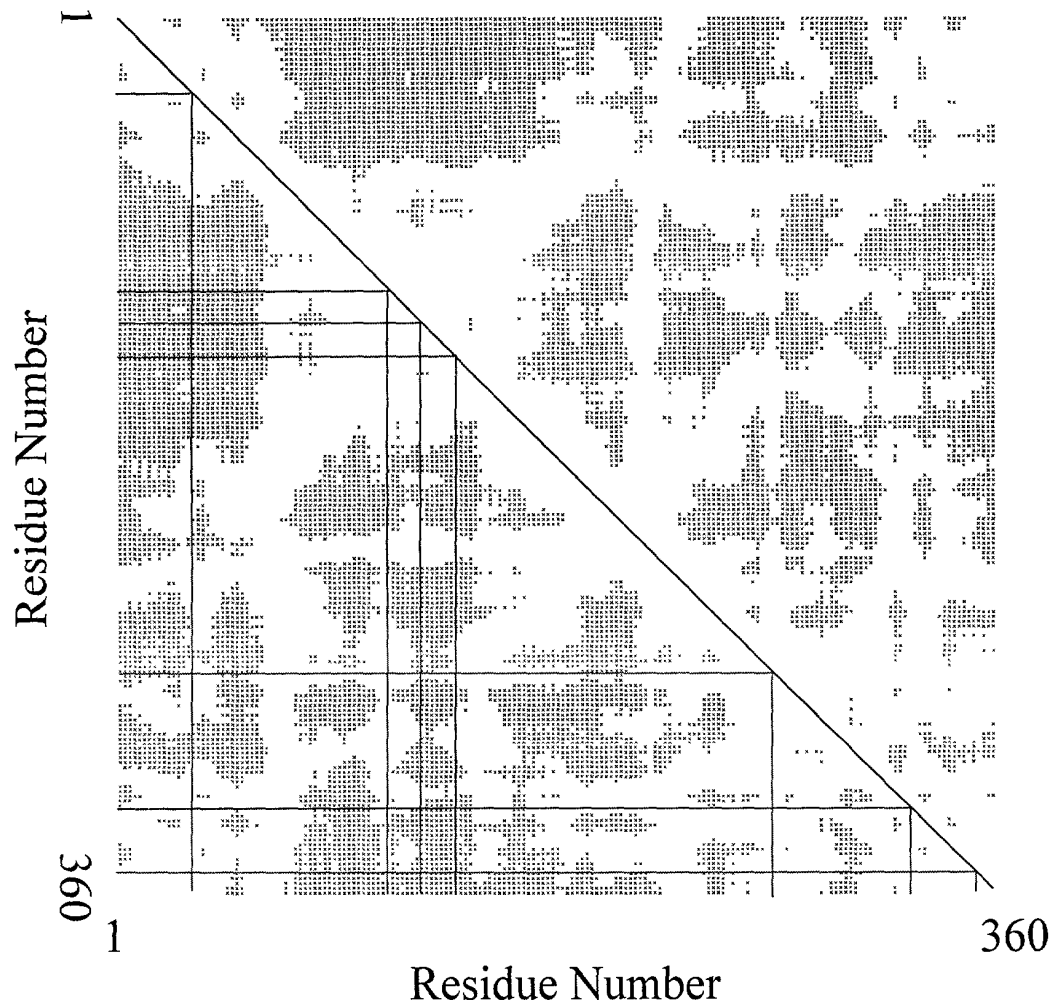
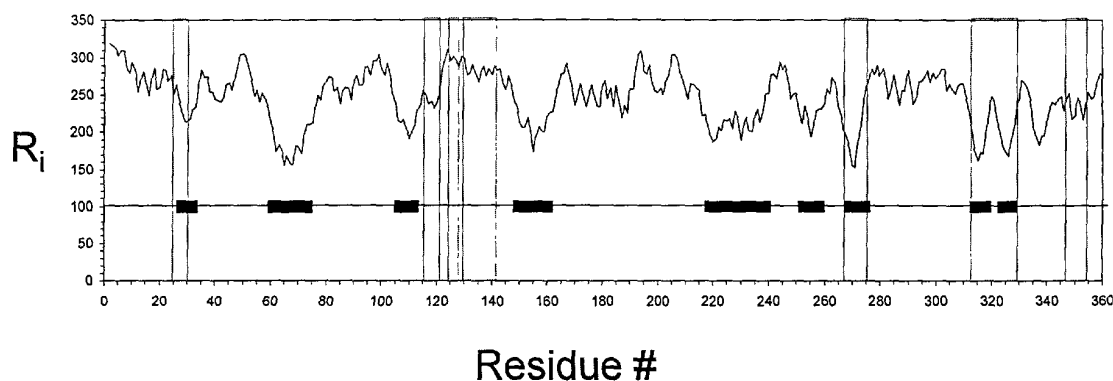
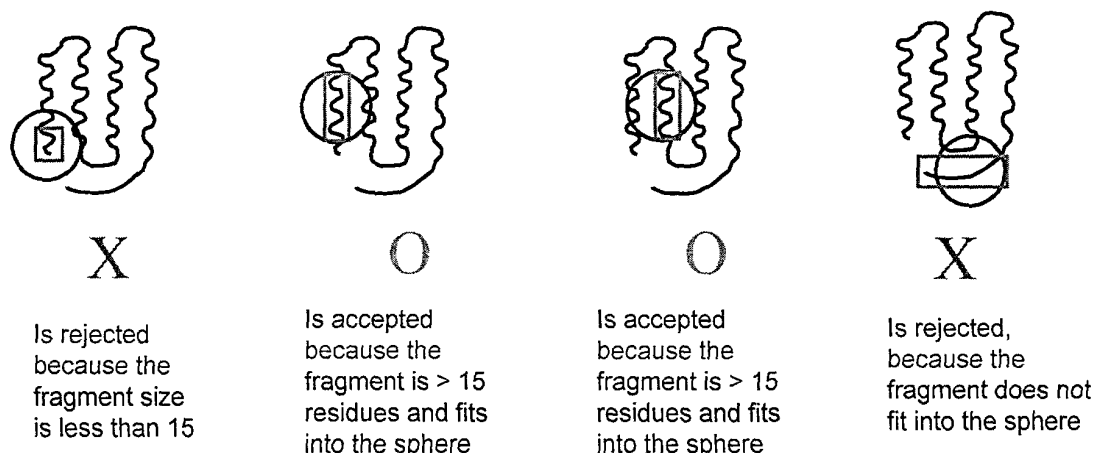


FIG. 21



**FIG. 22**



(1) Pick a sphere size (21 angstroms, like Go-Gilbert) and a disruption threshold; (2) Scan protein using segments at least the average number of residues for that sphere size or greater (e.g., >15 for 21 angstrom sphere); (3) Check the disruption of all the compact fragments identified in step 2. If the fragment has a disruption above a threshold value, keep it; otherwise, throw it out; (4) If the compact unit is disruptive, increment the schema disruption measure for all of the residues in the fragment by one. This indicates that crossovers within the fragment are disfavored.

**FIG. 23**

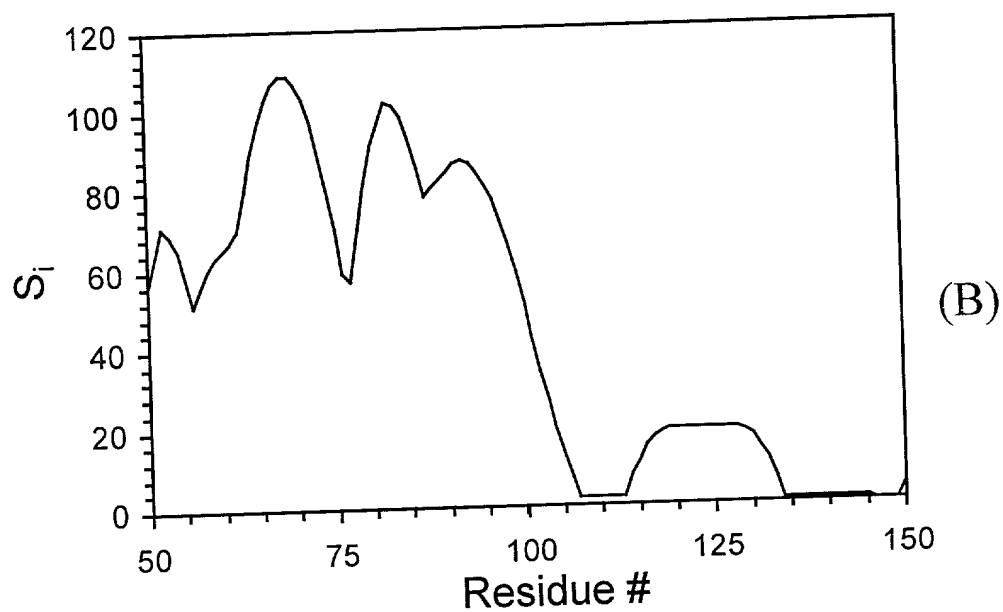
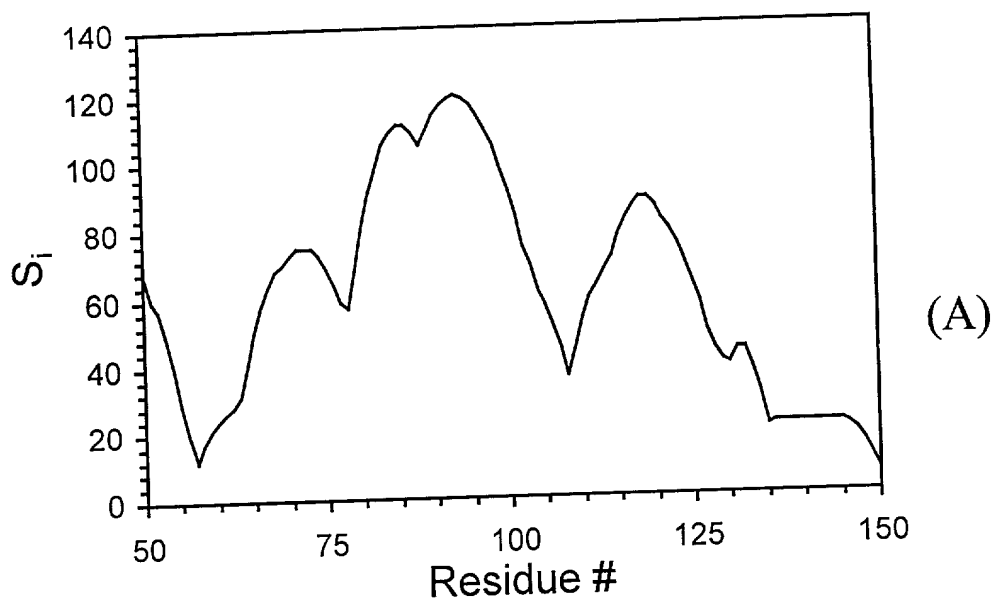


FIG. 24

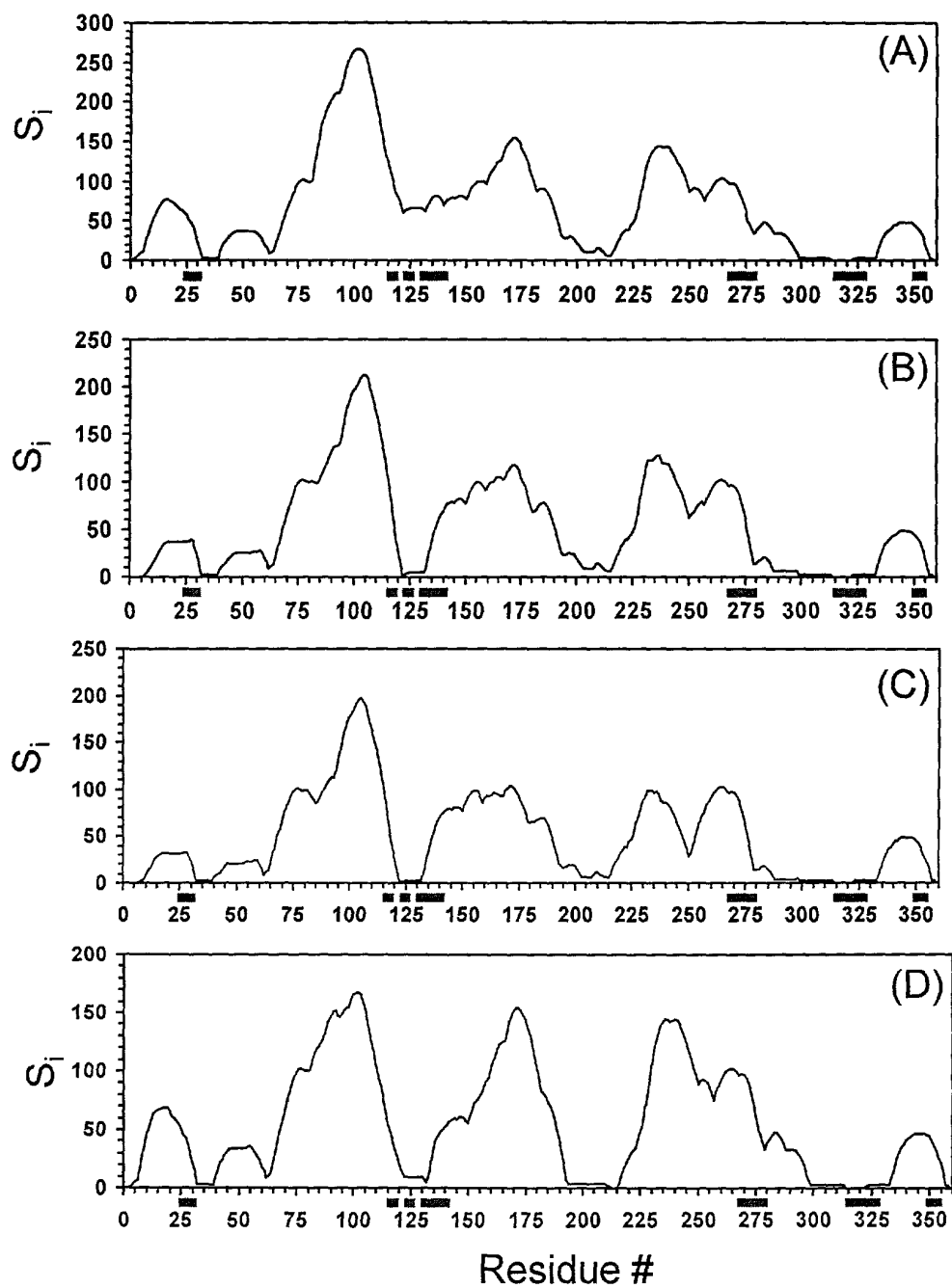


FIG. 25

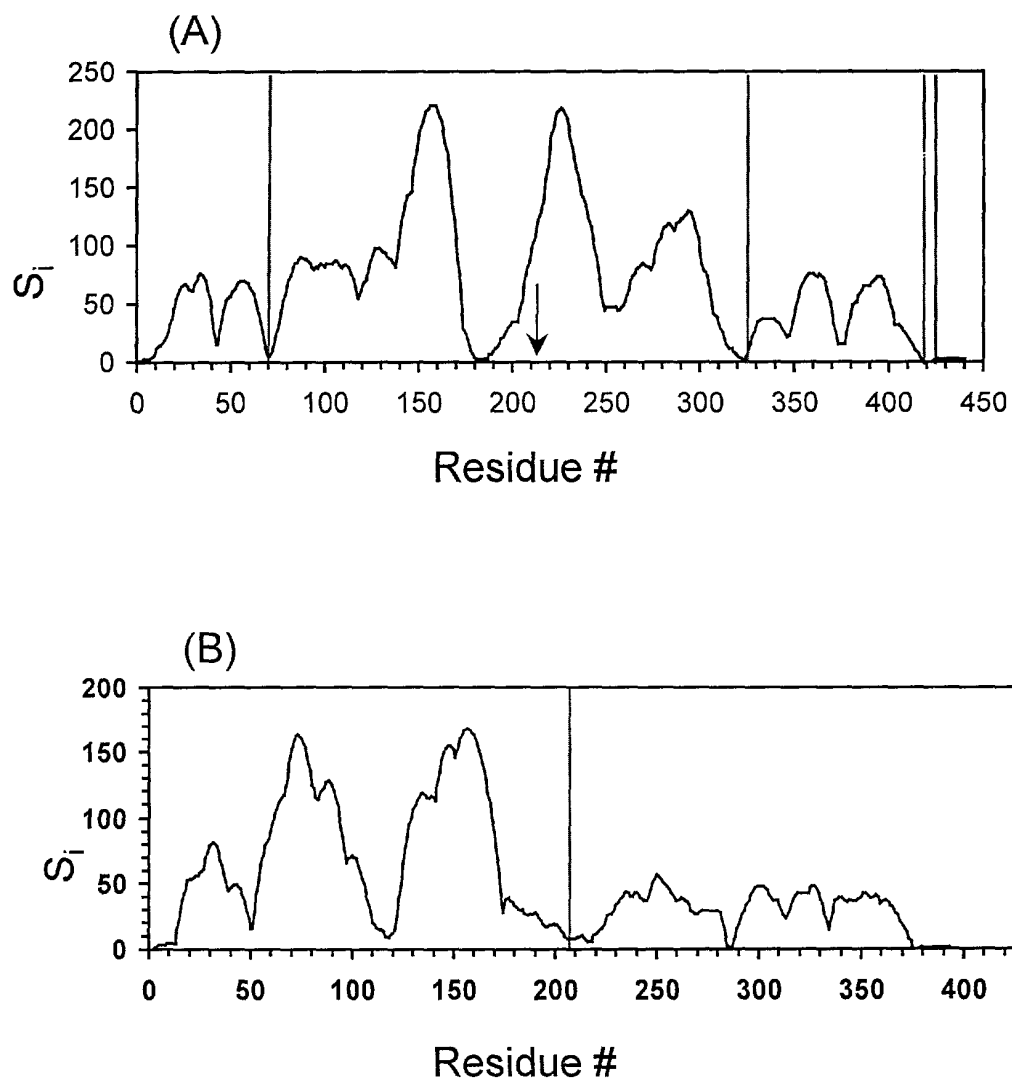
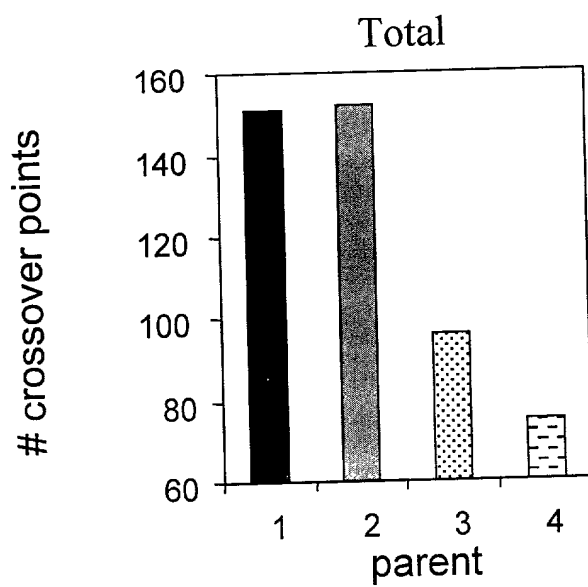
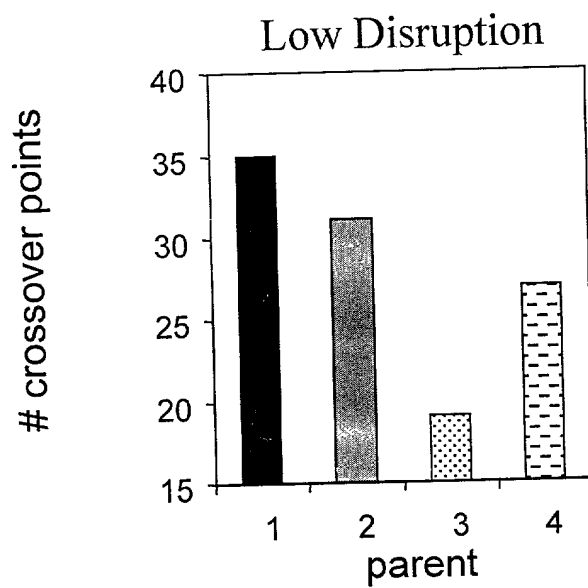


FIG. 26





**FIG. 27A**



**FIG. 27B**